Attachment H

Proposal # 2001-J-200 (Office Use Only)

PSP Cover Sheet (Attach to the front of each proposal)

Proposal Title: Genetic Identification of Watershed-Dependent Species of Special Concern in the Central Valley

Applicant Name: San Francisco State University
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Amount of funding requested: _____

Some entities charge different costs dependent on the source of the funds. If it is different for state or federal funds list below.

State cost $640,176 Federal cost: $851, 669

Cost share partners? ____**Yes** __**X**__**No**

Identify partners and amount contributed by each_____

_____

Indicate the Topic for which you are applying (check only one box).

☐	Natural Flow Regimes	☐	Beyond the Riparian Corridor
☐	Nonnative Invasive Species	☐	Local Watershed Stewardship
☐	Channel Dynamics/Sediment Transport	☐	Environmental Education
☐	Flood Management	☒	Special Status Species Surveys and Studies
☐	Shallow Water Tidal/ Marsh Habitat	☐	Fishery Monitoring, Assessment and Research
☐	Contaminants	☐	Fish Screens

What county or counties is the project located in? Entire CALFED region

What CALFED ecozone is the project located in? See attached list and indicate number. Be as specific as possible ALL Zones #1-14 (see figure 1)

Indicate the type of applicant (check only one box):

☐	State agency	☐	Federal agency
☐	Public/Non-profit joint venture	☐	Non-profit
☐	Local government/district	☐	Tribes
☒	University	☐	Private party
☐	Other: _____		

Indicate the primary species which the proposal addresses (check all that apply):

- ☐ San Joaquin and East-side Delta tributaries fall-run chinook salmon
- ☐ Winter-run chinook salmon
- ☐ Spring-run chinook salmon
- ☐ Late-fall run chinook salmon
- ☐ Fall-run chinook salmon
- ☐ Delta smelt
- ☐ Longfin smelt
- ☐ Splittail
- ☐ Steelhead trout
- ☐ Green sturgeon
- ☐ Striped bass
- ☐ White Sturgeon
- ☐ All chinook species
- ☐ Waterfowl and Shorebirds
- ☐ All anadromous salmonids
- ☒ Migratory birds
- ☐ American shad
- ☒ Other listed T/E species: Reptiles/Amphibians

Indicate the type of project (check only one box):

- ☒ Research/Monitoring
- ☐ Watershed Planning
- ☐ Pilot/Demo Project
- ☐ Education
- ☐ Full-scale Implementation

Is this a next-phase of an ongoing project? Yes ___ No_X_

Have you received funding from CALFED before? Yes ___ No_X_

If yes, list project title and CALFED number_____

Have you received funding from CVPIA before? Yes ___ No_X___

If yes, list CVPIA program providing funding, project title and CVPIA number (if applicable):

By signing below, the applicant declares the following:

- The truthfulness of all representations in their proposal;
- The individual signing the form is entitled to submit the application on behalf of the applicant (if the applicant is an entity or organization); and
- The person submitting the application has read and understood the conflict of interest and confidentiality discussion in the PSP (Section 2.4) and waives any and all rights to privacy and confidentiality of the proposal on behalf of the applicant, to the extent as provided in the Section.

Bruce Macher, Associate Dean, Research

Printed name of applicant

Bruce A. Macher

Signature of applicant

Environmental Compliance Checklist

All applicants must fill out this Environmental Compliance Checklist. Applications must contain answers to the following questions to be responsive and to be considered for funding. *Failure to answer these questions and include them with the application will result in the application being considered nonresponsive and not considered for funding.*

1. **Do any of the actions included in the proposal require compliance with either the California Environmental Quality Act (CEQA), the National Environmental Policy Act (NEPA), or both?**

   _____ YES __X__ NO

2. **If you answered yes to # 1, identify the lead governmental agency for CEQA/NEPA compliance.**

   _____
   Lead Agency

3. **If you answered no to # 1, explain why CEQA/NEPA compliance is not required for the actions in the proposal.**

   This project is research only; PIs hold all applicable permits.

4. **If CEQA/NEPA compliance is required, describe how the project will comply with either or both of these laws. Describe where the project is in the compliance process and the expected date of completion.**

5. **Will the applicant require access across public or private property that the applicant does not own to accomplish the activities in the proposal?**

   __X__ YES For Field Sampling at sites TBD _____ NO

   If yes, the applicant must attach written permission for access from the relevant property owner(s). Failure to include written permission for access may result in disqualification of the proposal during the review process. Research and monitoring field projects for which specific field locations have not been identified will be required to provide access needs and permission for access with 30 days of notification of approval.

6. Please indicate what permits or other approvals may be required for the activities contained in your proposal. Check all boxes that apply.

LOCAL		
Conditional use permit	___	
Variance	___	
Subdivision Map Act approval	___	
Grading permit	___	
General plan amendment	___	
Specific plan approval	___	
Rezone	___	
Williamson Act Contract cancellation	___	
Other _____ (please specify)		
None required	___	
STATE		
CESA Compliance	___	(CDFG)
Streambed alteration permit	___	(CDFG)
CWA § 401 certification	___	(RWQCB)
Coastal development permit	___	(Coastal Commission/BCDC)
Reclamation Board approval	___	
Notification	___	(DPC, BCDC)
Other:	X	Collecting permits (CDFG)
None required	___	
FEDERAL		
ESA Consultation	___	(USFWS)
Rivers & Harbors Act permit	___	(ACOE)
CWA § 404 permit	___	(ACOE)
Other :	X	Collecting Permits (USFWS)
None required	___	

DPC = Delta Protection Commission
CWA = Clean Water Act
CESA = California Endangered Species Act
USFWS = U.S. Fish and Wildlife Service
ACOE = U.S. Army Corps of Engineers

ESA = Endangered Species Act
CDFG = California Department of Fish and Game
RWQCB = Regional Water Quality Control Board
BCDC= Bay Conservation and Development Comm.

Land Use Checklist

All applicants must fill out this Land Use Checklist for their proposal. Applications must contain answers to the following questions to be responsive and to be considered for funding. *Failure to answer these questions and include them with the application will result in the application being considered nonresponsive and not considered for funding.*

1. Do the actions in the proposal involve physical changes to the land(i.e. grading, planting vegetation, or breeching levees) or restrictions in land use (i.e. conservation easement or placement of land in a wildlife refuge)?

   _____ YES ____X___ NO

2. If NO to # 1, explain what type of actions are involved in the proposal (i.e., research only, planning only).

   Research Only

3. If YES to # 1, what is the proposed land use change or restriction under the proposal?

4. If YES to # 1, is the land currently under a Williamson Act contract?

   _____ YES _____ NO

5. If YES to # 1, answer the following:

   Current land use _____
   Current zoning_____
   Current general plan designation _____

6. If YES to #1, is the land classified as Prime Farmland, Farmland of Statewide Importance or Unique Farmland on the Department of Conservation Important Farmland Maps?

   _____ YES _____ NO _____ DON'T KNOW

7. If YES to # 1, how many acres of land will be subject to physical change or land use restrictions under the proposal?
   _____

8. If YES to # 1, is the property currently being commercially farmed or grazed?

   _____ YES _____ NO

9. If YES to #8, what are the number of employees/acre_____
   the total number of employees _____

10. Will the applicant acquire any interest in land under the proposal (fee title or a conservation easement)?

_____	__X__
YES	NO

11. What entity/organization will hold the interest?_____

12. If YES to # 10, answer the following:

Total number of acres to be acquired under proposal	_____
Number of acres to be acquired in fee	_____
Number of acres to be subject to conservation easement	_____

13. For all proposals involving physical changes to the land or restriction in land use, describe what entity or organization will:

manage the property	_____
provide operations and maintenance services	_____
conduct monitoring	_____

14. For land acquisitions (fee title or easements), will existing water rights also be acquired?

_____	_____
YES	NO

15. Does the applicant propose any modifications to the water right or change in the delivery of the water?

_____	_____
YES	NO

16. If YES to # 15, describe_____

GENETIC IDENTIFICATION OF MANAGEMENT UNITS FOR WATERSHED-DEPENDENT SPECIES OF SPECIAL CONCERN

EXECUTIVE SUMMARY

Land-use decisions involving watersheds often require trade-offs with respect to which areas to conserve, develop, or restore. When threatened species are involved, setting priorities can become very complex. Here we propose the use of molecular genetic information in order to better identify and prioritize populations and watersheds for conservation and management.

An essential precursor to managing species is the ability to identify the spatial scale at which they should be managed. Most Central Valley species of special concern have fragmented distributions stemming from alterations of the landscape, whether natural (e.g., flooding rivers) or human-induced (e.g., urbanization or agriculture). These patterns of fragmentation have important management implications. Without knowing how populations are connected (both demographically and genetically) it is impossible to identify the spatial scale at which they should be managed. Some remnant populations may represent unique genetic units and be high conservation priorities; alternatively some small populations at risk of extirpation may be genetically similar to larger, stable populations elsewhere and therefore represent lower conservation priority. Because demographically independent populations should be managed as separate units, accurate identification of these units is essential.

Molecular-genetic techniques have been shown to be of great utility in identifying population units for management and have been proven to provide essential data for making informed conservation decisions. We propose to use two complementary molecular markers to elucidate the genetic structure of Central Valley populations and to identify corresponding management units for eight species of conservation importance in the Central Valley: three amphibians, one reptile, and four passerine songbirds. We will use the resulting genetic information to propose management units for these watershed-dependent species. Species were selected both for their conservation importance and their preference for critical habitats, including Riparian, Grasslands, Wetlands, Vernal Pools, and Aquatic habitats. Due to the breadth of organisms targeted, the study will offer a model for determining management units across taxa – both aquatic and terrestrial – in the Central Valley. Specifically, by taking a cross-species approach we hope to identify concordant patterns that will aid managers in making informed conservation decisions that maximize the biodiversity of the region as a whole.

The proposed research is a cross-disciplinary effort requiring expertise in both molecular genetics and the field ecology and natural history of our target taxa. To this end we have assembled an accomplished group of scientists with relevant expertise from three institutions: San Francisco State University, the University of California-Davis, and the Point Reyes Bird Observatory.

PROJECT DESCRIPTION

PROBLEM AND CONCEPTUAL MODELS

An essential precursor to managing species that depend on riparian habitat and waterways is to identify the spatial scale at which they should be managed. Most Central Valley species of special concern have fragmented geographic distributions stemming either from natural disjunctions in population structure or, more commonly, from anthropogenic causes. Patterns of fragmentation have important management implications. Genetically isolated sets of populations may exhibit strikingly different demographic trends, and may differ greatly in their levels of connectivity. The complementary concepts of Evolutionary Significant Units (ESUs), Management Units (MUs) and metapopulation dynamics address this issue from different perspectives, but all are based on the fundamental understanding that evolutionarily isolated populations exist in demographic isolation, whereas metapopulations exist as interconnected sets of subpopulations with strong demographic connections. Because demographically independent populations should be managed as separate units (Moritz, 1994), accurate identification of those units is essential (Moritz, 1994, Smith and Wayne 1996, Shaffer et al., 2000).

The primary goal of this study is to test competing hypotheses about the relative importance of geographic distance versus geographic barriers in producing population structure, and to then use the resulting genetic information to propose management units for a set of eight watershed-dependent vertebrate species of concern. Many disjunct or fragmented populations of Central Valley species exhibit an unknown level of connectivity with other such populations. Defining management units by following the movement of individuals by radio-tracking or using mark-recapture techniques is often impractical or impossible, particularly over a broad geographic scale (e.g. Trenham et al. in press; Trenham et al., submitted). An alternative, powerful, and efficient method of identifying population structure is to assay genetic markers that vary on both macro- and micro- geographic scales. Such genetic markers provide a means of determining the boundaries of local populations, assessing the genetic distinctiveness of populations, and linking the regional population dynamics of local populations. Genetic markers can thereby inform management decisions by providing critical information on such demographic issues as source-sink dynamics and metapopulation structure.

The use of molecular markers has become a standard method for the examination of population structure, and genetic techniques have provided essential data for making highly informed conservation decisions (Smith and Wayne 1996, Avise and Hamrick 1996; Smith et al. 1993, Girman et al., 1993; Edwards, 1993; Quinn, 1992; Wayne et al., 1994; Wenink et al., 1994; Zink and Dittman, 1993, Shaffer et al., 2000). Importantly for threatened species, the application of genetic markers to large-scale assessment programs has been fostered by recent technological advances that allow DNA to be obtained non-destructively from sources such as toe-clips or feathers (Taberlet and Bouvet, 1991; Morin and Woodruff, 1996), and that allow the rapid and efficient genotyping of hundreds of individuals.

We propose to use two complementary molecular markers to elucidate the genetic structure and to identify corresponding management units for eight species of conservation importance in the Central Valley: three amphibians, one reptile, and four passerine songbirds (see Figure 1 for current sampling locations). Our molecular approach includes the analysis of mitochondrial DNA (mtDNA) control region sequence and the analysis of hypervariable di- or tetra-nucleotide repeat regions (microsatellites). Such genetic markers are powerful management tools because they provide essential information on patterns of recent demographic exchange, information particularly important for assessing the effects of anthopogenic changes on demographic processes and the ability of a species to recover from environmental perturbation and maintain viable populations in areas where habitat restoration is underway (Nur and Sydeman 1999). Population specific demographic information is also needed to link local and regional population declines with their underlying causes such as pollutants, deforestation, fragmentation, and land use trends (Hartshorn, 1992; Terborgh, 1992; James and McCulloch, 1995; Davidson and Shaffer, in press). Furthermore,

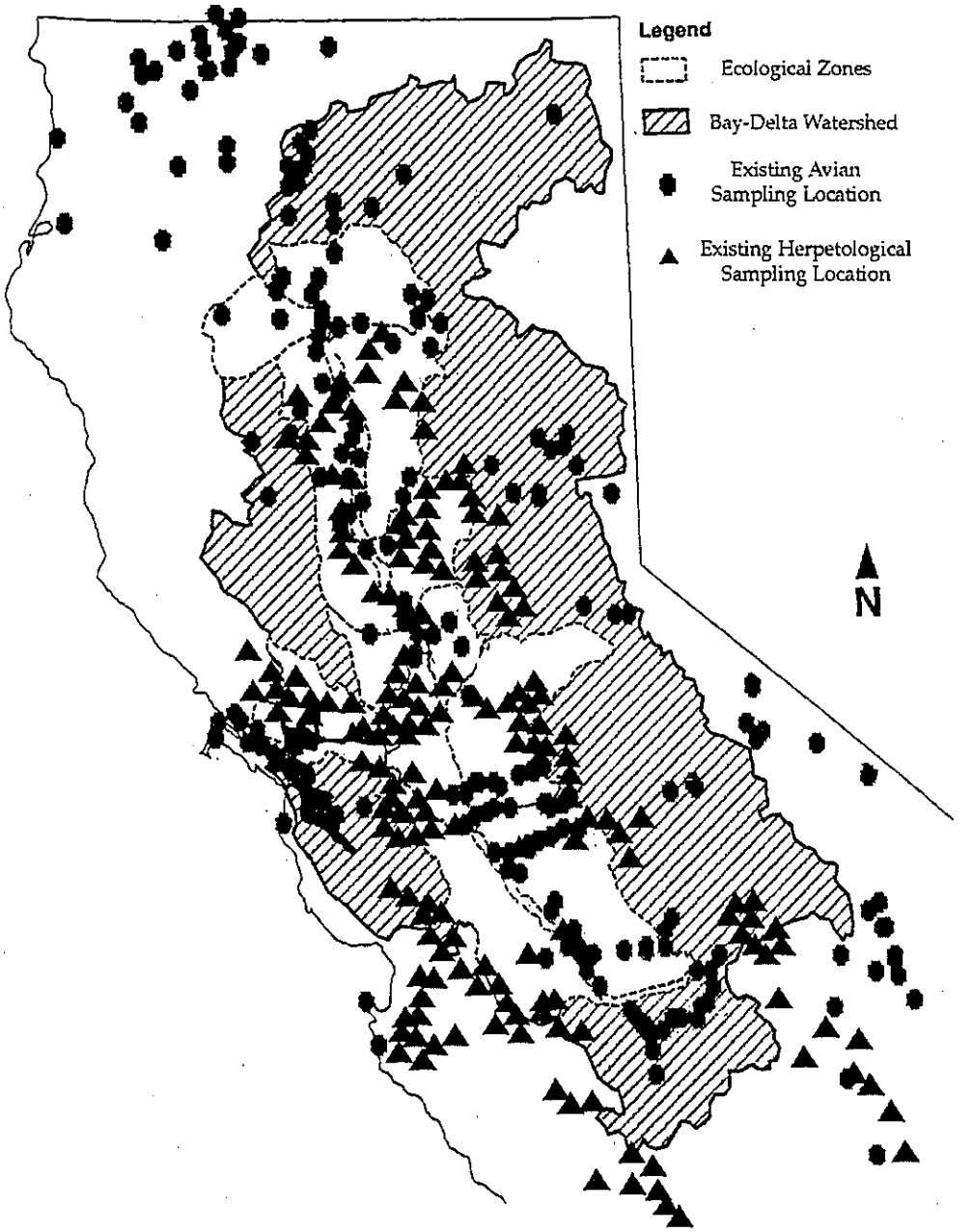

Figure 1. Locations of existing sampling stations for genetic samples and/or demographic monitoring of the eight focal amphibian, reptile, and bird species.

our study of important indicator species will serve as a model for the establishment of Management Units for a range of taxa across California's Central Valley.

Specific Objectives

To characterize spatial population structure in Central Valley amphibians, reptiles, and songbirds to provide resource mangers with information linking population structure with demographic processes. Specifically, we propose :

1) to characterize the boundaries and connectivity of distinct population units in three Central Valley amphibian species, one reptile, and four riparian songbird species;

2) to test whether Central Valley populations show a pattern of "isolation by distance," and, if so, to determine whether distance should be measured as watercourse distance or as straight-line distance. These data will allow us to test competing hypotheses about the relative roles of geographic distance and demographic connectivity (such as dispersal along or between drainages, or between fragments of riparian habitat) in determining Central Valley management units.

3) to assess whether subsets of these amphibian, reptile, and songbird species exhibit congruent patterns of genetic variation such that they could be managed in concert, and also validate a strategy for investigating management units in other Central Valley taxa with similar distributions and dispersal capabilities; and ultimately

4) to provide explicit recommendations on which local populations should be considered distinct Management Units. The form of these recommendations will depend upon the type of genetic structure found in each species. For example, if population structure tracks drainage patterns rather than simple geographic distance among sampling sites, then the restoration and maintenance of particular riparian corridors will be important for species management. If distance or dispersal barriers better defines Management Units, then habitats connecting these areas must be maintained to ensure the longer-term viability of these species. For all species, these recommendations will also incorporate information on demographic processes and anthropogenenic stressors from ongoing monitoring projects.

Study Species

Amphibians and Reptiles: Amphibians and reptiles are generally low-vagility animals with highly subdivided populations and deep genetic differentiation over small spatial scales (Shaffer and Breden, 1989; Shaffer et al, 2000). We have chosen our four target species (one reptile, three amphibians) because 1) they represent the extremes of how these animals interact with the aquatic landscape of the Central Valley and adjacent watersheds, and 2) they are exemplars of the overall trend of declining amphibians and reptiles in the Central Valley (Fisher and Shaffer, 1996; Jennings and Hayes, 1994). Two of the species are found primarily in flowing streams and associated riparian habitats, while the other two are found in vernal pools/ponds of the Central Valley and surrounding foothills. Taken together, our target species are primary inhabitants of several key habitats associated with the Central Valley project, including Riparian, Grasslands, Wetlands, Vernal Pools, and Central Valley Aquatic Habitats.

Western Pond Turtle, *Clemmys marmorata*: Historically distributed in most Pacific slope drainages throughout California, Oregon and southern Washington, *C. marmorata* is now informally considered endangered or threatened over all of its range (Jennings and Hayes, 1994), although it is not currently listed under the Endangered Species Act. *Clemmys marmorata* is the only native freshwater turtle in California, and is considered a Species of Special Concern by the California Department of Fish and Game. It is restricted to slow-moving riverine systems, including low-gradient streams with appropriate adjacent upland habitat for basking and egg-laying (Holland, 1991a). *Clemmys marmorata* still occurs in most streams and rivers from sea level to 1430 m elevation (Holland, 1992). Identification of management units for this highly impacted species is particularly critical since juvenile recruitment appears to be low to non-existent in many areas, and remedying this situation

requires intensive on-site management of nesting sites, including nest predators (Jennings and Hayes, 1994). Because the Western Pond Turtle is restricted to riverine habitats, but travels many hundreds of meters to nest and overwinter (Storer, 1930), it is unclear whether gene flow occurs exclusively along river channels, or whether overland movement leads to genetic interchange of populations across drainage systems. Preliminary population genetic results based on mitochondrial DNA sequence data (Janzen, Hoover and Shaffer, 1997) and DNA fingerprinting (Gray, 1995) detected relatively little genetic differentiation among pond turtle populations, although some differentiation was found between Sacramento river and San Joaquin drainage populations (Janzen, Hoover and Shaffer, 1997). It seems clear that rapidly-evolving microsatellite loci will be necessary to define management units in this critically-sensitive species.

Foothill yellow-legged frog, *Rana boylii*. This stream and river-dwelling species was once common in foothill and mid-elevation watersheds below 1800 m throughout California, but currently has declined over a significant portion of its range (Jennings 1996). It is still present in most populations, although numbers of animals are often reduced from previous levels. *Rana boylii* is considered to be endangered/threatened in some parts of its range (Jennings and Hayes, 1994), and is considered to be a Species of Special Concern by the state of California throughout its range. Although reasons for declines have yet to be fully documented, water diversions and dams affect downstream habitats for frog breeding and tadpole rearing as well as creating unsuitable habitats (reservoirs) upstream that may act as barriers to dispersal (Lind et al. 1996). Because it is completely restricted to flowing stream habitats, *R. boylii* is a candidate for deep genetic differentiation among drainages with relatively little overland gene flow. Preliminary data for populations from several coast range and Sierra foothills populations indicate that significant genetic variation exists in the mitochondrial cytochrome *b* gene (Shaffer, unpublished data), although we currently have no idea how that variation is structured within and among streams and creeks. Based on our preliminary data, both mitochondrial DNA and microsatellite loci should provide a clear picture of population substructure and management units in this species.

California Tiger Salamander, *Ambystoma californiense*. This species is a vernal pool/pond endemic, restricted to the Central Valley, foothills up to about 1000 m elevation, and the inner coast range from Sonoma to Santa Barbara counties. It has been a candidate for listing under the Endangered Species Act since 1992, and is treated as a Species of Special concern by the state of California. Populations identified as a genetically-defined management unit from Santa Barbara county by the Shaffer lab were protected under an emergency listing by the USFWS in April, 2000. We are completing a very large (82 populations, approximately 700 individual salamanders) survey of *A. californiense* from throughout its range for the mitochondrial DNA control region, and are using this data set to define management units for both state and federal agency biologists. Although our mitochondrial DNA demonstrates strong patterns of differentiation among some units, particularly from Sonoma and Santa Barbara counties, it appears to be evolving too slowly for clear diagnosability of populations from the main Central Valley portion of the range. Some hints of geographic substructure exist, particularly between inner coast range population units from San Francisco to Monterey and the remainder of the species, suggesting that multiple management units may well exist within the main bulk of the species. Given the extreme interest in vernal pool inhabitants in general, and of this species in particular (Jennings and Hays, 1994; Barry and Shaffer, 1994; Shaffer and McKnight, 1996), we consider the complete delineation of management units with microsatellite loci to be critical in this species. It will also provide us with a clear set of results on patterns of genetic differentiation among populations of a low-vagility, vernal pool endemic that can be used to generate predictions for co-distributed species of plants, invertebrates, and amphibians, many of which are threatened or endangered.

Western Spadefoot, *Scaphiopus hammondi*. This species of frog is the most extreme vernal pool specialist in the Central Valley region, and is largely co-distributed with the California

Tiger Salamander. The species is considered to be threatened by Jennings and Hayes (1994), and is a designated Species of Special Concern by the State of California. Because of its very rapid larval development time (about three weeks, Feaver, 1971), *S. hammondi* occupies smaller, and more ephemeral vernal pools than *A. californiense*, although it is also found in large vernal pool complexes. The genetic relationships of populations of this species are completely unknown, leading Jennings and Hayes (1994, p. 96) to state that "The biggest gap in current understanding of *S. hammondi* relates to its population structure.." We view the Western Spadefoot as particularly important, since it provides the opportunity to quantify management units of an endemic species that utilizes both large vernal pools (and thus replicates the California Tiger Salamander) and small, ephemeral vernal pools that are critical to many plant and crustacean populations. Although we have accumulated tissue samples from a broad spectrum of sites across the range of this species (about 80 sites and over 1000 individuals), no molecular work has yet been done on the species. We envision using both mitochondrial DNA and microsatellite loci to quantify population substructure in the western spadefoot.

Birds: Riparian bird species breeding in the Central Valley region have experienced striking population declines and extirpations (Gaines 1977, Griggs and Small 2000). This region has undergone drastic land use changes in the past 150 years; 90% of the historic riparian habitat in the Central Valley is now gone, adversely affecting the many bird species that are reliant on this productive habitat. Habitat degredataion in the Central Valley has particularly affected riparian species (e.g., the Least Bell's Vireo, Yellow-billed Cuckoo, and Yellow-breasted Chat). The species that remain are fragmented to different degrees, but the effect of this fragmentation on patterns of demographic exchange among local populations is unknown.

Three of our avian target species are Neotropical migrants dependent on riparian habitat and one is a colonial, marsh-nesting blackbird that is endemic to the Central Valley. These species are the subjects of past and continuing study (partially supported by CALFED) by the PRBO at a number of sites Central Valley watershed, and therefore extensive population-specific demographic information is available on them. The demographic data indicate that source-sink dynamics are a critical consideration for effective management (e.g., see Yellow Warbler description below). All four taxa have all been designated focal species of interest by the Riparian Habitat Joint Venture and the California chapter of Partners in Flight., and recent studies have demonstrated that their presence is a reliable indicator of riparian health and therefore a good gauge of restoration success (Nur et al., in press; Griggs and Small, in prep.; see also the Riparian Conservation Plan of the Riparian Habitat Joint Venture at www.prbo.org).

Yellow Warbler, *Dendroica petechia*. Broadscale mtDNA-based surveys (Klein and Brown, 1996; Lovette and Bermingham, 1999) have found substantial differentiation among Yellow Warbler populations, and microsatellite surveys have identified extensive allelic diversity within local populations elsewhere in North America (Dawson et al. 1997). The Yellow Warbler is of special interest owing to its dramatic decline over the past century and local extirpation along much of the mainstem of the Sacramento River (Griggs and Small, in prep). The Yellow Warbler is also extirpated from the San Joaquin River. Though historically Yellow Warblers were common throughout the Central Valley (Grinnell & Miller 1944) and large populations persist at middle elevations in the watershed, at present there is but a tiny remnant population along the Sacramento River meander belt. This population experiences high nest predation rates, suggesting that either the population will soon go extinct, or that its existence is maintained by immigration from surrounding regions. Genetic information on the extent to which such 'rescue effects' are necessary for maintaining viable populations of riparian species is information of crucial management importance. The genetic information provided by this study will also assess whether the remnant meander belt population is a distinct genetic entity.

Common Yellowthroat, *Geothlypis trichas*. This species is a California Species of Special Concern. Numbers are thought to be greatly reduced compared to historical levels, though it is still found as a breeder in both the Sacramento and San Joaquin River valleys. It nests primarily

in tall emergent wetland vegetation, but will also nest in tall emergent upland vegetation. It prefers marshy areas, as well as early successional, shrubby riparian habitat.

Black-headed Grosbeak, *Pheucticus melanocephalus*. Currently, nothing is known about the micro- or macro-scale genetic diversity of this species. The Black-headed Grosbeak is of special management interest because statistical analyses by the Point Reyes Bird Observatory (Geupel et al. 1996) indicate that this species has low productivity coupled with high adult survival. This is an unusual demographic pattern among migratory songbirds, one in which dispersal and philopatry may exert a particularly strong influence on local population dynamics, and in turn, on genetic structure. Population numbers appear stable. Nests are often located in willow, alder, box elder, or ash, and the species prefers semi-open canopy with moderate shrub cover (Griggs and Small, in prep.).

Tricolored Blackbird, *Agelaius tricolor*. The Tri-colored Blackbird is one of only two bird species endemic to the Central Valley and adjacent coastal California. Owing to its restricted distribution, it is a species of conservation importance and is listed as a California Species of Special Concern, but no information is presently available on the genetic diversity of this species. Abundance of this species has declined by more than 80% since the 1930's, with losses of colonies in the Sacramento and San Joaquin River valleys accounting for much of this decline. Tricolored Blackbirds breed in large, dense aggregations, but their breeding colonies move from year to year. Colonies are especially dense in the Central Valley. They prefer marshy areas with emergent cattails and tules. Successful nesting requires proximity to productive sources of insect food and protection from nest predators.

Analytical Approach and Hypotheses

Our basic strategy is to collect and analyze population samples that will allow us to simultaneously address three questions for each species:

Question I. Is the entire Central Valley watershed a single or multiple ESUs? As a consequence of historical isolation, populations may have different "evolutionary potential" from one another (Moritz, 1994). Genetic identification of these Evolutionary Significant Units (ESUs) is most often based on mitochondrial DNA (mtDNA) divergence (Moritz, 1994).

For amphibians and reptiles, we will develop PCR primers for the mtDNA control region, and use a combination of direct sequencing and outgroup heteroduplex to score 10-20 individuals per population. Using a similar strategy, our ongoing analysis of *A. californiense* has led to the recognition of at least three ESUs within this species, two of which (Sonoma and Santa Barbara counties) are critically imperiled. The USFWS has emergency listed the Santa Barbara tiger salamander, and we are now developing on-site management strategies with the goal of providing sufficient habitat for all genetically defined units so that the species can be de-listed, and landholders can continue with responsible land stewardship and use in the future.

The greater dispersal capabilities of avian species mean that it is less likely that the deep mtDNA divergences characterizing ESUs will be found at the geographic scale of the Central Valley. However, our work on other migratory songbirds has revealed that ESUs can be detected on a larger geographic scale (Mila et al., 2000, Kimura et al., in prep), and the known morphological variation (and associated subspecific distinction) within some of our focal taxa suggest that the Central Valley might contain multiple ESUs of some avian species. We have already developed and optimized the necessary avian mtDNA PCR primers for use in avian population screening. To determine whether more than one ESU is present within each species, we will screen multiple individuals from all widely separated sampling sites using mtDNA. However, if the more likely scenario of a single Central Valley ESU holds, then management efforts should target demographic enhancements on a regional scale, rather than the preservation of genetic biodiversity on a population-specific basis.

Question II: What is the relationship between genetic divergence and geographic distance among sites for each species? We propose to examine this question at two spatial scales, to

evaluate two distinct management goals. First, we ask what the relationship is between genetic and geographic distance over a spatial scale of tens to hundreds of kilometers within each species. For this question, we will use both mtDNA and more rapidly evolving microsatellite loci, and the samples collected for goal #1 above. If no significant variation is found within an ESU, then we would argue that there are no clear management units within each ESU, leading to management of sensitive species and reserve design at the ESU level. If significant substructure is found, then more localized, on-site management is the strategy of choice. We will also examine this at a fine spatial scale, since amphibian and reptile populations can be very finely substructured with little apparent gene flow among local populations whereas birds may show a pattern of isolation-by-distance. For this aspect of the work, we will identify three replicate regions (ESUs if they exist, or geographic regions if they do not) for each species, and collect tissue samples at 1-10km intervals along replicated transects. Based on our previous work, amphibian and reptile populations at such a spatial scale can be significantly differentiated--for example, in the montane toad *B. canorus,* populations in Yosemite and Kings Canyon National Parks were significantly differentiated over 2-5 km distances (Shaffer et al., 2000), implying that very little natural gene flow occurs among ponds, and that management should be at the individual pond level. Alternatively, based on mark-recapture data, the salamander *A. californiense* has high levels of migration among ponds over a five km distance in the inner coast range (Trenham et al., in press; Trenham et al., in revision), suggesting that individual breeding sites are less important than maintaining a few, large complexes of vernal pools in a region. In birds, fine-scale differentiation has been found for some taxa but not others (Brawn et al., 1996; Smith et al., 1997; Lovette et al., 1999b; Clegg, 2000).

For birds, the availability of detailed, species-specific demographic data will also allow us to link patterns of genetic population structure with local and regional demographic trends. The requisite demographic data are already in-hand and will be analyzed following Nur et al. (1999). Superimposing complementary genetic and demographic information on population connectivity will provide a unique, powerful, and management-relevant perspective on regional population dynamics and on the likely longer-term viability of existing remnant populations.

Question III: Are river drainages significant MUs for each species? Because all of our target species are intimately linked to riparian, wetland, and/or vernal pool habitats, one potential structuring force is individual river drainages. However, before watersheds are used to identify potential management units, we consider it to be a critical empirical question to quantify levels of genetic differentiation within and between drainages. Again, both mtDNA and microsatellite data will contribute to this analysis: Due to differences in life histories, mtDNA is likely to be most informative for amphibians and reptiles and microsatellites for birds. The information used to address questions I and II will provide an initial perspective on whether these taxa exhibit drainage-specific patterns of differentiation. To address this question more intensively, we will also choose a subset of three adjacent watersheds and collect five equidistant samples along each watershed from each amphibian and reptile species, and from the two bird species (Common Yellowthroat and Black-headed Grosbeak) with amenable distributions. This highly structured sampling scheme will allow us to partition the genetic variation among the set of 15 sites into variation within and among watersheds, holding straight-line distance among sites roughly constant. In a similar analysis of watershed properties in the toad *B. canorus,* we found that watersheds were the primary structuring agents among ponds in the relatively mesic Yosemite region, but not for the drier Kings Canyon drainages (Shaffer et al., 2000). Based on the biology of our amphibian and reptile target species, we predict that the importance of drainage systems in defining management units should increase with increasing reliance on flowing water habitats, leading to *R. boylii* > *C. marmorata* > *A. californiense* = *S. hammondi* for the importance of drainages in defining management units. For birds, we anticipate that the species with the greatest philopatry and adult survival (*P. melanocephalus* > *G. trichas*) will show the largest degree of drainage-specific structure in microsatellite frequencies.

FIELD SAMPLING

All samples will be collected under the appropriate state and federal permits, which are already in hand by the PIs as part of ongoing field studies.

Amphibians and Reptiles: For each of our four amphibian/reptile species, we will collect from a minimum of 10 populations from the east side of the Central Valley (from 100-1000 m elevation), 10 populations from the west side of the Central Valley, 10 from the coast range (both west and east flowing drainages) and 10 from the Valley proper (less than 50 m elevation, grassland vernal pool or riverine habitat) for a total of 40 populations. These samples will provide the large-scale sampling necessary to evaluate the existence of ESUs at the landscape level of the entire Central Valley watershed. We will collect tissue samples from a minimum of 10 individuals per locality and a maximum of 50 individuals, based on simulation studies indicating that this is the optimal sample size for microsatellite analysis (Gaggiotti et al., 1999). We have already completed field sampling for the salamander *A. californiense* (> 80 populations, ~2000 individuals) and the turtle *C. marmorata* (> 50 populations, ~3000 individuals), and have at least half of the necessary samples for the two anurans *S. hammondi* (~30 populations, 600 individuals) and *R. boylii* (15-20 populations, 350 individuals). Additionally, fine-scale targeted samples will be collected to address the specific hypotheses outlined above. We will identify three replicate regions (ESUs if they exist, or geographic regions if they do not) for each species, and collect tissue samples for ~ 20 sites at approximately 1-10 km intervals for a detailed analysis of geographic structure and isolation-by-distance. We will also choose three adjacent watersheds for each species and collect five equidistant samples along each watershed. This sampling scheme will allow us to partition the genetic variation among the set of 15 sites into variation within and among watersheds, holding airline distance among sites roughly constant.

Birds: Owing to the restricted habitat affinities and anthropogenically fragmented distributions of our focal bird species, our sampling will necessarily target remnant populations on a species-specific basis. We anticipate sampling feathers from at least 15-20 birds from at least 10 sites spanning the full Central Valley range of each species. All samples will be taken from locally breeding individuals (i.e., excluding migrating individuals passing through an area) or young from a known site. Sampling of Tri-colored Blackbirds depends on the availability of colonies (there may be fewer than 10 that can be sampled), however , a large number of samples can be obtained from each colony.

LABORATORY TECHNIQUES AND ANALYSES

We will collect data from both mtDNA and nuclear microsatellite markers for all eight focal species. The basic techniques for DNA extraction and amplification via the polymerase chain reaction are well-established in the Shaffer and Smith labs for a variety of species, including seven of our eight target species or their close allies (Shaffer and McKnight, 1996; Janzen et al., 1997; Shaffer et al., 1997; Smith et al., 1997; Lovette et al., 1998; 1999a; Lovette and Bermingham 1999; Schneider et al., 1999; Shaffer et al., 2000; Shaffer et al., in prep; Shaffer, unpublished). We have worked with several segments of the mtDNA, including the non-coding and highly variable control region, the protein-coding cytochrome *b* gene, several tRNA genes, the non-coding control region, and a newly-discovered intergenic spacer. Our combined experience strongly indicates that for intraspecific work of the kind proposed here, the control region and associated spacers are the regions of choice, and we will work with them in all cases. For population-level screens of hundreds to thousands of individuals, the Shaffer lab has pioneered the use of both single-strand conformation polymorphisms (SSCP, Shaffer et al., 2000) and outgroup heteroduplex analysis (OGH, Shaffer et al., unpublished). These techniques are relatively rapid screening procedures that allow us to examine small (SSCP) and quite large (up to 800 base-pair fragments for OGH) DNA fragments quickly and efficiently. Because it works on much larger fragments, we currently favor OGH, and will use this technique to initially screen samples for each species. We recently finished a large analysis of over 700 California Tiger Salamander samples with this technique, and found it to extremely reliable, sometimes at the single base pair level.

Microsatellite loci will certainly be the molecules of choice for examining fine-scale population subdivision in amphibians and reptiles and are likely to be the most informative markers for birds on a more regional scale, particularly among populations that may be exchanging individuals at the present time. The use of microsatellite loci requires the development of species-specific microsatellite libraries; to reduce cost and increase efficiency, we will use a commercial vendor to create and screen libraries. We will then process samples in-house and run our then in ABI 377 automated DNA sequencers (available at both UC Davis and SFSU). By multiplexing three loci per lane per gel, microsatellites can be processed with highly efficient and cost-effective throughput.

MtDNA sequence data will be analyzed both via an analysis of variance approach modified for molecular sequence data, to deduce the significance of the geographic divisions among local and regional population groupings (Excoffier et al., 1992; Chenoweth et al., 1998), and using standard tree-based phylogenetic methods (e.g., parsimony and maximum-likelihood). Population subdivision at microsatellite loci will be assessed by quantifying differences in allele frequency distributions between sampling sites. Pairwise comparisons of allele frequency differences between sampling sites will be conducted using a Monte Carlo approximation of Fisher's exact test in GENEPOP version 3.1 (Raymond and Rousset, 1999). The magnitude of any population subdivision will also be quantified via assignment tests (Paetkau et al., 1995) using the GENEPOP software. In this test, each individual is assigned to the sampling to which it has the greatest likelihood of belonging, by comparing the genotype profile of the individual with the observed allele frequency distributions of each sampling site. The percentage of correct assignments for each sampling site can then be used as an indicator of the level of distinctiveness within each region.

Expected Products and Data Accessibility

The results and implications of these studies will be presented in a number of complementary formats to target maneagement, scientific, and public constituencies. Our quarterly and annual reviews to CalFed will also be copied to the Upper Sacramento River Advisory Council, the Wetlands Ecosystem Goal Project, and NGOs associated with the PRBO, and will be posted on the WWW. We plan to present our work at both national scientific meetings and resource maneagement symposia, and to publish our completed results both in scientific journals and as explicit reports for resource managers. All nucleotide sequences will be permanently archived in publically-accessible databases (e.g., GenBank). Microsatellite frequency data will be included in the CalFed and published reports.

Work Schedule and Project Timetable

Years One and Two

Field Work—in each of the first two years, field teams from PRBO and UC Davis will conduct an intensive sample collection program targetting multiple populations of each focal taxon.
Lab Work—Molecular techinques (including microsatellite libraries, which can be generated from samples already in-hand) will be developed during the first 18 months and screening of populations will commence as soon as the first field season is completed.

Year Three

Lab Work—Molecular genetic analysis of remaining samples will be completed.

Data Analysis and Management Recommendations—Computer-based population genetic and phylogenetic analyses will be completed for all populations of all taxa. Manuscripts will be prepared for submission to scientific journals, and management reports for each species prepared for CalFed.

APPLICABILITY TO CALFED ERP GOALS AND IMPLEMENTATION PLAN AND CVPIA PRIORITIES

In the current proposal, we lay out a strategy for identifying areas in the Central Valley of maximum importance with respect to the preservation of eight important watershed-dependent terrestrial species. The species we propose to study, like many other species of special concern in the Central Valley, have fragmented distributions. Without knowing how populations are connected (both demographically and genetically) it is impossible to identify the spatial scale at which they should be managed. Some remnant populations may represent unique genetic units and be high conservation priorities; alternatively some small populations at risk of extirpation may be genetically similar to larger, stable populations elsewhere and therefore represent lower conservation priority. Our genetic techniques allow us to both identify unique units and assess levels of interchange between populations. This information on genetic and demographic processes can be directly incorporated into management plans to foster the most rapid recovery of these species. Importantly, by taking a cross taxonomic approach we hope to identify possible concordant patterns across taxa that will aid managers in making informed conservation decisions that maximize diversity across multiple taxa.

1. ERP Goals

Our proposal directly addresses three of the CALFED Ecosystem Restoration Program Goals:

GOAL #1: At-Risk Species

The species targeted are minimally designated as Species of Special Concern by the California Department of Fish and Game (see Study Species section for endangered/threatened classifications). The proposed project will identify management units of these important, at-risk, species. Identification of these units is a critical first step toward achieving recovery of these species, so that they can be removed from at-risk lists. Ultimately, the results of this project may serve as a model for establishing management units for species from varied taxonomic groups (e.g., fish, mammals).

GOAL #2: Ecosystem Processes and Biotic Communities

By identifying management units using gnetic markers we will help develop priority-setting strategies that preserve both the pattern of biodiversity in the Central Valley *and* the diversification process. Typically, priority-setting efforts to preserve particular species, give the greatest weight to areas where the species are found in greatest density. In the current proposal, we seek to identify particular populations of our target species with the greatest conservation value: i.e., those exhibiting the greatest genetic distinctiveness. By preserving these distinct, diverse units, conservation efforts will succeed in preserving the biotic process that will enable the species to survive future threats.

GOAL #4: Habitats

Efforts to protect or restore functional habitat types throughout the Central Valley depend on the ability to establish priorities among and within these habitats. All of our target species are primary inhabitants of key habitats in the Central Valley: Riparian, Grasslands, Wetlands, Vernal Pools, and Aquatic Habitats. By identifying management units for the eight target species, the project will indicate priority areas of conservation (as well as areas of less importance).

2. Relationship to Other Ecosystem Restoration Projects

The current proposal will play an important role in informing current CALFED-funded projects. For example, our findings would provide important information to the overall Watershed "Action Plan" effort, as well as smaller-scale efforts like the Silver Creek Watershed Management and Action Plan, and the Nature Conservancy's Site-Specific Management Planning on the Sacramento River project.

QUALIFICATIONS

The proposed research is an integrated analysis of population genetics, phylogenetics and demographics in representative terrestrial vertebrate groups at selected sites in California's Central Valley. It is a cross-disciplinary effort requiring expertise in molecular genetics and the ecology of the central valley's birds and reptiles/amphibians.

San Francisco State University – PI Thomas Smith

Dr. Thomas Smith (team leader), has over two decades of experience in evolutionary ecology and molecular genetics as applied to natural populations. Currently Smith is Professor of Biology at SFSU and is a faculty member in the Center for Population Biology at UC Davis. He has extensive experience leading multi-institutional, cross-disciplinary projects, including a current NSF-funded, $2.6 million, four-university effort to study worldwide rainforest biodiversity .

Dr. Smith earned his doctorate in Zoology at UC-Berkeley in 1988, where he remained forthree years as a postdoctoral researcher and Senior Fulbright Fellow. He is an elected fellow of the California Academy of Sciences (since 1994) and a senior fellow of the Zoological Society of London (since 1997). While his research has taken him to five continents, Dr. Smith remains committed to California conservation efforts, including service on various U.S. Fish and Wildlife Service recovery teams of endangered bird species and service on the PRBO board of directors. Combining molecular genetics with ecological expertise, Dr. Smith has more than 70 publications, including a dozen in conservation genetics. Relevant publications are highlighted in the literature cited section.

Other key SFSU personnel:
Dr. Sonya Clegg, a recent graduate of U. of Queensland (Australia), and Dr. Irby Lovette, a recent graduate of the U. of Pennsylvania, bring molecular genetics expertise to the project. Both have a backround in avian molecular genetics, with previous and current work spanning population to species level studies. Dr. Clegg will be at SFSU through November 2002 and Dr. Lovette will be at SFSU through September 2001.

The Point Reyes Bird Observatory – PI Nadav Nur and co-PI Geoffrey Geupel

The Point Reyes Bird Observatory (PRBO) is a non-profit, membership organization founded in 1965 with the mission to use science to conserve birds and their environment. PRBO leads various terrestrial research projects including: monitoring migratory birds in the Sacramento River National Wildlife Refuge; an assessment of songbird conservation in California's Riparian Habitats; a tidal marsh bird project in the San Francisco Bay Region; and an assessment of bird abundance and diversity in the Redwood Creek Watershed.

PRBO efforts to conduct bird sampling in the current proposal will be led by:
Nadav Nur, Ph.D., Director of Population Ecology ,who has analyzed results from PRBO's long term research and monitoring programs for landbirds and seabirds. Dr. Nur's Research interests include developing and applying new statistical methods to the study of bird demography and development of population models to study the impact of toxic spills on bird populations. Dr. Nur is the author of over 45 peer-reviewed scientific articles and book chapters concerning avian population biology. Relevant publications are highlighted in the literature cited section.

Geoffrey Geupel, Director of Terrestrial Research and California Partners-in-Flight co-chair, whose current objective is to implement a habitat-based songbird monitoring program, to assist land managers in reversing population declines throughout the west. Mr. Geupel has been Principal Investigator on numerous projects concerning riparian birds throughout California.

University of California-Davis: PI Brad Shaffer

Dr. Brad Shaffer, Professor at the Center for Population Biology, studies the evolution and ecology of amphibians and reptiles, with a strong focus at the interface of molecular population genetics, systematics, and conservation biology.. Dr. Shaffer's work has been extensively used by management agencies at both the state and federal level to help manage the several sensitive species of amphibians, reptiles and fishes. Current laboratory work uses new technologies in genotype screening of both mitochondrial and microsatellite nuclear DNA markers to understand the phylogeography of species ranging from freshwater turtles in Australia, the Amazon basin and the U.S. to declining amphibians in California, In addition to his molecular work, Dr. Shaffer also conducts toxicological and ecological work to help identify mechanisms responsible for population declines of California amphibians. Thus, his work covers the full spectrum of activities from the identification of significant management units of amphibians and reptiles across the Central Valley watershed to empirical analyses of the factors responsible for declines, with the goal of providing solid, defensible strategies for protecting key population segments and reversing patterns of declines.

Dr. Shaffer earned his PhD in Evolutionary Biology from the University of Chicago in 1982, and pursued his postdoctoral research at the University of Wisconsin-Madison and the Field Museum of Natural History (Chicago). After two years on the UC-Irvine faculty, Dr. Shaffer moved to the Davis campus in 1988. In addition to his academic research, which has produced more than 50 peer-reviewed publications, Dr. Shaffer has served on the Declining Amphibian Task Force of the California/Nevada Workgroup, as well as the NBS/BRD Workgroup to Develop a National Amphibian Monitoring System (1994-present). Relevant publications are highlighted in the literature cited section.

BUDGET JUSTIFICATION

The current proposal seeks three years of funding to identify management units of 8 species of conservation importance in the central valley. The investigation is a collaboration of San Francisco State University, the University of California-Davis, and the Point Reyes Bird Observatory, with SFSU as the lead institution and UCD and PRBO operating on sub-contracts. General responsibilities will be:

SFSU: general oversight and all avian molecular work
PRBO: all avian field sampling
UCD: all amphibian work (both field sampling and molecular work)

Note that PRBO has only a two-year budget, as field sampling (both avian and amphibian) will be conducted only in years one and two of the three year project.

San Francisco State University

	Year One	Year Two	Year Three	Total
1) SENIOR PERSONNEL	$0	$0	$0	$0

Dr. Tom Smith will be the project director and will oversee all aspects of avian research. Dr. Smith's time is contributed.

2) OTHER PERSONNEL	$46,230	$48,542	$50,969	$145,741

A post-doctoral researcher (to be named) will undertake the avian molecular work, including the development of microsatellite libraries, DNA sequencing, and analysis. The post-doc will be a full-time researcher at the CSU-mandated annual salary of $33,500, plus benefits of 38%. An annual COLA (5%) is built in to years two and three.

Current senior SFSU post-docs Dr. Sonya Clegg and Dr. Irby Lovette will coordinate the avian genetic work at no cost to the project.

C. LABORATORY COSTS	$65,000	$25,000	$25,000	$115,000

Genetic analyses (DNA sequencing) will be performed in the SFSU Conservation Genetics Lab for the four target avian species, at $32 per sample (includes DNA extraction, marker amplification and double–stranded sequencing on ABI Prism 377 automated sequencer). For this purpose we seek $75,000, divided evenly over the three years (NB: the Smith Lab currently has sufficient samples of these species in a freezer allowing genetic work to begin immediately). In addition, we seek first-year funding for the development of four microsatellite libraries, at $10,000 per library (based on prior contracts with Genetic Identification Services, Inc. of Chatsworth, CA).

D. INDIRECT COSTS	(state)	$22,438	$12,778	$11,395	$46,611
	(federal)	$76,289	$43,445	$38,744	$158,478

If project funds are from a state source, the SFSU indirect cost rate of 15% will apply; if funds are federal, SFSU's federally-negotiated indirect cost rate of 51% will apply.

Sub-contract: Point Reyes Bird Observatory

	Year One	Year Two	Year Three	Total
A. SENIOR PERSONNEL	$1,688	$1,772	$0	$3,460

Dr. Nadav Nur, PRBO Population Ecology Program Director, and Geoffrey Geupel, PRBO Terrestrial Program Director, will oversee all avian field sampling. For each of the two years, 0.33 months of salary for Dr. Nur is requested, with additional time contributed. Mr. Geupel's salary is contributed. A 5% COLA is sought in year two.

B. OTHER PERSONNEL	$8,618	$9,049	$0	$17,667

Under the supervision of Mr. Geupel and Dr. Nur, two part-time (50% effort) field biologists will undertake avian field sampling for a four-month season in each of years one and two. A 5% COLA is sought in year two.

C. TRAVEL	$2,500	$2,625	$0	$5,125

Travel and field support for the sampling team in each of years one and two is requested.

D. FIELD SUPPLIES	$550	$578	$0	$1,128

We request nominal funding for general field supplies (e.g., mist nets, compasses, calipers, field books).

E. INDIRECT COSTS	$3,873	$4,067	$0	$7,940

Whether from federal or state sources, PRBO applies an indirect cost rate of 29%.

Sub-contract: University of California, Davis

	Year One	Year Two	Year Three	Total
A. SENIOR PERSONNEL	$0	$0	$0	$0

Dr. Brad Shaffer will oversee all aspects of reptile and amphibian research. Dr. Shaffer's time is contributed.

B. OTHER PERSONNEL	$53,900	$56,056	$38,938	$148,894

A post-doctoral researcher (to be named) will undertake the reptile/amphibian molecular work, including DNA sequencing, and analysis. Funding is sought for an annual salary starting at $30,000, plus benefits of 20%. An annual COLA (4%) is built in to years two and three.

A graduate research assistant (PhD student) will coordinate all reptile and amphibian field sampling for two years, with stipend/benefits of $11,300 in year one. The GRA will be assisted in the summer sampling seasons by two undergraduate field assistants, who will each receive stipend/benefits of $3,300 for two months. Second year salary/benefit levels reflect a 4% COLA.

C. TRAVEL $4,000 $4,000 $0 $8,000
Travel and field support for the three-person sampling team (GRA and two undergrads) for two months in each of years one and two is requested at $2000/month.

D. LABORATORY COSTS $36,000 $36,000 $36,000 $108,000
Genetic analyses (microsatellite libraries, DNA sequencing, etc.) will be performed in the Dr. Shaffer's Molecular Genetics Lab for the four target reptile and amphibian species. We seek $3000/month for this purpose.

E. OTHER $3,000 $3,120 $6,120
Graduate Research Assistant student fees of $3,000 annually (plus 4% anticipated increase in year two) are requested.

F. INDIRECT COSTS (state) $9,390 $9,606 $7,494 $26,490
(federal) $43,664 $46,107 $36,345 $126,116
If project funds are from a state source, the UCD indirect cost rate of 10% will apply to all direct costs (except student fees); if funds are federal, UCD's federally-negotiated indirect cost rate of approximately 48% (46.5% in year one; 48% in year two; 48.5% in year three) will apply to all direct costs (except student fees).

TOTAL BUDGET BY TASK

WITH FEDERAL INDIRECT COST										
YEAR	TASK	Salary	Benefits	Travel	Equipment	Supplies	Contracts	Other	IDC	TOTAL
YR 1	Lab Work	33,500	12,730				72,000	65,000	109,037	292,267
	Field Work						38,256		14,789	53,045
YR 2	Lab Work	35,175	13,367				73,440	25,000	78,025	225,007
	Field Work						39,760		15,594	55,354
YR 3	Lab Work	36,934	14,035				74,938	25,000	75,089	225,996
	Field Work									

WITH STATE INDIRECT COST										
YEAR	TASK	Salaries and Benefits		Travel	Equipment	Supplies	Contracts	Other	IDC	TOTAL
YR 1	Lab Work	33,500	12,730				72,000	65,000	29,480	212,710
	Field Work						38,256		6,221	44,477
YR 2	Lab Work	35,175	13,367				73,440	25,000	19,982	166,964
	Field Work						39,760		6,469	46,229
YR 3	Lab Work	36,934	14,035				74,938	25,000	18,889	169,796
	Field Work									

BUDGET DETAIL

San Francisco State University	**YR 1**	**YR 2**	**YR 3**	**TOTAL**
Personnel:				
Post Doctoral Research Associate				
$33,500/year (w/5% COLA)	$33,500	$35,175	$36,934	$105,609
Fringe Benefits 38%	$12,730	$13,367	$14,035	$40,132
Total SFSU Salaries	**$46,230**	**$48,542**	**$50,969**	**$145,741**
Laboratory Costs:				
Microsatellite Libraries (4 libraries @ $10,000)	$40,000			$40,000
DNA sequencing, etc.	$25,000	$25,000	$25,000	$75,000
Total Reagents	**$65,000**	**$25,000**	**$25,000**	**$115,000**
Total Direct Costs SFSU	**$111,230**	**$73,542**	**$75,969**	**$260,741**
Direct Costs, Sub-Contract: University of California, Davis	**$96,900**	**$99,176**	**$74,938**	**$271,014**
Direct Costs, Sub-Contract: Point Reyes Bird Observatory	**$13,356**	**$14,024**		**$27,380**
TOTAL DIRECT COSTS, PROJECT	**$221,486**	**$186,742**	**$150,907**	**$559,135**
Scenario A: IndirectCosts at State Rate				
Indirect Costs, Sub-Contract: UCD (10%)	$9,390	$9,606	$7,494	$26,490
Indirects Costs, Sub-Contract: PRBO (29%)	$3,873	$4,067	$0	$7,940
Indirect Costs, SFSU (15%)	$22,438	$12,778	$11,395	$46,611
TOTAL INDIRECT COSTS, PROJECT	**$35,701**	**$26,451**	**$18,889**	**$81,041**
TOTAL COSTS, PROJECT	**$257,187**	**$213,193**	**$169,796**	**$640,176**
Scenario B: Indirect Costs at Federal Rate				
Indirect Costs, Sub-Contract: UCD (46.5%-48.5%)	$43,664	$46,107	$36,345	$126,116
Indirect Costs, Sub-Contract: PRBO (29%)	$3,873	$4,067	$0	$7,940
Indirect Costs, SFSU (51%)	$76,289	$43,445	$38,744	$158,478
TOTAL INDIRECT COSTS, PROJECT	**$123,826**	**$93,619**	**$75,089**	**$292,534**
TOTAL COSTS, PROJECT	**$345,312**	**$280,361**	**$225,996**	**$851,669**

SUB-CONTRACT BUDGETS

UC DAVIS					
A.	**Personnel:**				
	Post Doctoral Research Associate	$30,000	$31,200	$32,448	$93,648
	Benefits	$6,000	$6,240	$6,490	$18,730
	Graduate Research Assistant	$10,971	$11,410		$22,381
	Benefits	$329	$342		$671
	Undergraduate Summer Assistants	$6,000	$6,240		$12,240
	Benefits	$600	$624		$1,224
	Total UCD Salaries	**$53,900**	**$56,056**	**$38,938**	**$148,894**
B.	**Travel:**				
	Field Work: Central Valley, CA				
	$2K/mo * 2 months/yr	$4,000	$4,000		$8,000
	Total UCD Travel	**$4,000**	**$4,000**	**$0**	**$8,000**
C.	**Laboratory Costs:**				
	DNA sequencing, etc.	$36,000	$36,000	$36,000	$108,000
	Total Laboratory Costs	**$36,000**	**$36,000**	**$36,000**	**$108,000**
D.	**Other**				
	Grad Student Fees	**$3,000**	**$3,120**		**$6,120**
	Total Direct Costs UC-Davis	**$96,900**	**$99,176**	**$74,938**	**$271,014**
Scenario A: State Funds					
	State Indirects (10%)	$9,390	$9,606	$7,494	$26,490
	Total Costs, UC-Davis	**$106,290**	**$108,782**	**$82,432**	**$297,504**
Scenario B: Federal Funds					
	Federal Indirects (46.5%, 48%, 48.5%)	$43,664	$46,107	$36,345	$126,116
	Total Costs, UC-Davis	**$140,564**	**$145,283**	**$111,283**	**$397,130**

POINT REYES BIRD OBSERVATORY					
A.	**Personnel:**				
	Principal Investigator (0.33 months/yr)	$1,688	$1,772		$3,460
	Field Biologists	$8,618	$9,049		$17,667
	Total PRBO Salaries	**$10,306**	**$10,821**	**$0**	**$21,127**
B.	**Travel:**				
	Field Work: Central Valley, CA	2500	$2,625		$5,125
	Total Travel	**$2,500**	**$2,625**	**$0**	**$5,125**
C.	**Field Supplies**				
	Mist nets, etc.	$550	$578		$1,128
	Total Supples	**$550**	**$578**	**$0**	**$1,128**
	Total Direct Costs UC-Davis	**$13,356**	**$14,024**	**$0**	**$27,380**
Scenario A/B: State/Federal Funds (IDC same in both scenarios)					
	Indirects (29%)	$3,873	$4,067		$7,940
	Total Costs, PRBO	**$17,229**	**$18,091**		**$35,320**

LITERATURE CITED (publications by PI's and co-PI's are shown in bold)

Avise JC and Hamrick JL. 1996. Conservation Genetics: case histories from nature. Chapman and Hall, New York.

Barry SJ and Shaffer HB. 1994. The status of the California Tiger Salamander (*Ambystoma californiense*) at Lagunita: A fifty-year update. Journal of Herpetology 28:159-164.

Brawn JD, Collins TM, Medina M and Bermingham E. 1996. Associations between physical isolation and geographical variation within three species of Neotropical birds. Molecular Ecology 5:33-46.

Chenoweth SF, Hughes JM, Keenan CP, Shane L. 1998 Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, *Lates calcarifer* (Australian barramundi). Heredity 80:187-197.

Clegg S. 2000. Evolutionary divergence of island silvereyes (*Zosterops lateralis*): the role of founder events, drift and selection. PhD Thesis, University of Queensland, Brisbane, Australia.

Davidson C, Shaffer HB and Jennings MR. In Press. Declines of the California red-legged frog: Spatial analysis of the climate change, UV-B, and pesticide hypotheses. Ecological Applications.

Dawson RJG, Gibbs HL, Hobson KA and Yezerinac SM. 1997. Isolation of microsatellite DNA markers from a passerine bird, *Dendroica petechia* (the yellow warlber), and their use in population studies. Heredity. 79:506-514.

Edwards SK. 1993. Mitochondrial gene genealogy and gene flow among island and mainland populations of a sedentary songbird, the Grey-crowned Babbler (*Pomatostomus temporalis*). Evolution. 47:1118-1137.

Excoffier L, Smouse P and Quattro J. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Feaver PE. 1971. Breeding pool selection and larval mortality of three California amphibians: *Ambystoma tigrinum californiense* Gray, *Hyla regilla* Baird and Girard, and *Scaphiopus hammondii* Girard. MA thesis, Fresno State College, Fresno, CA.

Fisher RN and Shaffer HB. 1996. The decline of amphibians in California's Great Central Valley Conservation Biology. 10:1387-1397.

Gaines DF. 1977. The valley riparian forests of California: Their importance to bird populations. Pp 57-85. *In* Ann Sands (editor) Riparian Forests in California: Their ecology and conservation. Institute of Ecology Publication 15, Univ. of California, Davis , CA.

Girman DJ, Kat PW, Mills MGL, Ginsberg J, Fanshawe J, Fitzgibbon C, Borner M, Wilson V, Laurenson K, and Wayne RK. 1993. Molecular genetic and morphologic analyses of the African wild dog (*Lycaon pictus*). Journal of Heredity 84:450-459.

Griggs T and Small S. In prep. Riparian Vegetation White Paper for CALFED. Draft of 27 March 2000. 64 pages. Available from tgriggs@jps.net or_ssmall@prbo.org.

Grinnell J and Miller AH. 1944. The distribution of the birds of California. Pacific Coast Avifauna 27:1-608.

Hartshorn GS. 1992. Forest loss and future options in Central America. In: Ecology and conservation of Neotropical migrant landbirds (Hagan and Johnston, eds.) Washington: Smithsonian Institution Press.

Holland DC. 1991. A synopsis of the ecology and status of the western pond turtle (*Clemmys marmorata*) in 1991. Report prepared for the United States Fish and Wildlife Service, National Ecology Research Center, San Simeon Field Station, San Simeon, CA.

Holland, DC 1992. Level and pattern in morphological variation: A phylogeographic study of the western pond turtle (*Clemmys marmorata*). Ph.D. Dissertation, University of Southwestern Louisiana, Lafayette, Louisiana.

Janzen FJ, Hoover SL and Shaffer HB. 1997. Molecular phylogeography of the western pond turtle (*Clemmys marmorata*): Preliminary results. Chelonian Conservation and Biology 2:623-626.

Jennings MR and Hayes MP. 1994. Amphibian and reptile species of special concern in California. California Department of Fish and Game, Rancho Cordova, CA.

Jennings MR. 1996. Status of amphibians. Pp. 921-944 *in*: Sierra Nevada Ecosystem Project: Final Report to Congress, vol. II, Assessments and scientific basis for management options. Davis: University of California, Centers for Water and Wildland Resources.

James FC, and McCullogh CE. 1995. The strength of Inferences about causes of trends in populations. In: Ecology and Management of Neotropical Migratory Birds (Martin and Finch, eds.) New York, Oxford University Press.

Kimura M. In prep. Admixture of breeding lineages of Wilson's warblers on wintering grounds. Master Thesis. Department of Biology, San Francisco State University.

Klein NK and Brown WM. 1994. Intraspecific molecular phylogeny in the Yellow Warbler (*Dendroica petechia*), and implications for avian biogeography in the West Indies. Evolution. 48:1914-1932.

Lovette IJ and Bermingham E.1999. Explosive speciation in the New World Dendroica warblers. Proceedings of the Royal Society of London: Biological Sciences 266:1629-1636.

Lovette IJ, Bermingham E, Rohwer S and Wood C. 1999a. Mitochondrial RFLP and sequence variation among closely related avian species and the genetic characterization of hybrid *Dendroica* warblers. Molecular Ecology 8:1431-1441.

Lovette IJ, Bermingham E and Ricklefs RE. 1999b. Mitochondrial DNA phylogeography and the conservation of endangered Lesser Antillean Icterus Orioles. Conservation Biology. 13:1088-1096.

Lind AJ, Welsh Jr. HH , and Wilson RA. 1996. The effects of a dam on breeding habitat and egg survival of the foothill yellow-legged frog (*Rana boylii*) in northwestern California. Herpetological Review 27:62-67.

McKnight ML and Shaffer HB. 1997. Large, rapidly evolving intergenic spacers in the mitochondrial DNA of the salamander family Ambystomatidae (Amphibia: Caudata). Molecular Biology and Evolution, 14:1167-1176.

Milá B. Girman D, Kimura M and Smith TB. 2000. Evidence for Pleistocene effects on the phylogeography of MacGillivary's warbler. Proceedings of the Royal Society of London: Biological Sciences. 267:1-8.

Morin, PA, and DS Woodruff. 1996. Noninvasive genotyping for vertebrate conservation. In: Molecular Genetic Approaches in Conservation (Smith and Wayne eds.) Oxford University Press.

Moritz C. 1994. Applications of mitochondrial DNA analysis in conservation; a critical review. Molecular Ecology 3:401-412.

Nur N and Sydeman WJ. 1999. Demographic processes and population dynamic models of seabirds: Implications for conservation and restoration. Current Ornithology 15:149-188.

Nur N, Jones SL, and Geupel GR. 1999. A Statistical Guide to Data Analysis of Avian Monitoring Programs. Biological Technical Publication, US Fish & Wildlife Service, BTP-R6001-1999.

Nur N, Geupel GR, and Ballard G. In press. Impact of the Cantara spill on abundance, diversity, productivity and survival of riparian birds. In press in Fish & Game (Calif. Department of Fish & Game).

Paetkau D, Calvert W, Sterling I and Strobeck C. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology 4:347-354.

Quinn, TW. 1992. The genetic legacy of mother goose - phylogenetic patterns of lesser snow goose *Chen caerulescens caerulescens* maternal lineages. Molecular Ecology 1:105-117.

Raymond M and Rousset F. 1999. GENEPOP: population genetics software for exact tests and ecumenicism. Version 3.2.

Schneider CJ, Smith TB, Larison B and Moritz C 1999. A test of alternative models of diversification in tropical rainforests: ecological gradients versus rainforest refugia. Proceedings of the National Academy of Sciences 96:13869-13873.

Shaffer HB and Breden F. 1989. The relationship between allozyme variation and life history: Non-transforming salamanders are less variable. Copeia 1989(4):1016-1023.

Shaffer HB and McKnight ML. 1996. The polytypic species revisited: genetic differentiation and molecular phylogenetics of the tiger salamander (*Ambystoma tigrinum*) (Amphibia: Caudata) complex. Evolution 50:417-433.

Shaffer HB, Fellers GM, Magee A and Voss SR. 2000. The genetics of amphibian declines: population substructure and molecular differentiation in the Yosemite Toad, *Bufo canorus* (Anura, Bufonidae) based on single-strand conformation polymorphism analysis(SSCP) and mitochondrial DNA sequence data. Molecular Ecology 9:245-257.

Smith TB, Bruford MW, and Wayne RK. 1993. The preservation of process: the missing element of conservation programs. Biodiversity Letters1:164-167.

Smith TB and Wayne RK. 1996. Molecular Genetic Approaches in Conservation. Oxford University Press.

Smith TB, Wayne RK, Girman D and Bruford MW. 1997. A Role for Ecotones in Generating Rainforest Biodiversity. Science 276:1855-1857.

Storer TI. 1930. Notes on the range and life history of the Pacific freshwater turtle, *Clemmys marmorata*. University of California Publications in Zoology 30(16):443-452.

Taberlet P and Bouvet J. 1991. A single plucked feather as a source of DNA for bird genetic studies.

Terborgh J. 1992. Perspectives on the conservation of Neotropical migrant landbirds. In: Ecology and Conservation of Neotropical Migrant Landbirds (Hagen and Johnston eds.) Washington: Smithsonian Institution Press.

Trenham PC, Shaffer HB, Koenig WD and Stromberg MR. In press. Long-term demographic variation in the California Tiger Salamander (*Ambystoma californiense*). Copeia.

Trenham PC, Koenig WD and Shaffer HB. Spatially autocorrelated demography and interpond migration in the California tiger salamander (*Ambystoma californiense*). In revision, Ecology.

Wayne RK, Bruford MW, Girman DJ, Rebholz WER, Sunnucks P, and Taylor AC. 1994. Molecular genetics of endangered species. In: Creative Conservation: Interactive Management of Wild and Captive Animals (Mace, Feiftnern and Olney, eds.), Chapman and Hall.

Wenink, PW, Baker AJ, and Tilanus MG. 1994. Mitochondrial control region sequences in two shorebirds species, the Turnstone and the Dunlin, and their utility in population genetic studies. Molecular Biology and Evolution 11:22-31.

Zink, RM and Dittmann DL. 1993. Gene flow, refugia, and evolution of geographic variation in the song sparrow (*Melospiza melodia*). Evolution 47:717-729.

OMB Approval No. 0348-0043

APPLICATION FOR FEDERAL ASSISTANCE

2. DATE SUBMITTED: 15-May-00	Applicant Identifier	
3. DATE RECEIVED BY STATE: N/A	State Application Identifier	
4. DATE RECEIVED BY FEDERAL AGENCY	Federal Identifier	

1. TYPE OF SUBMISSION:

Application	Preapplication
[ ] Construction	[ ] Construction
[X] Non-Construction	[ ] Non-Construction

5. APPLICANT INFORMATION

Legal Name: **SAN FRANCISCO STATE UNIVERSITY**

Organizational Unit: **Office of Research and Sponsored Programs**

Address (give city, county, state, and zip code): **1600 HOLLOWAY AVENUE, SAN FRANCISCO, CA 94132**

Name and telephone number of the person to be contacted on matters involving this application (give area code): Thomas B. Smith (415) 338-1089, Bruce A. Macher (415) 338-7091

6. EMPLOYER IDENTIFICATION NUMBER (EIN): 9 3 - 1 1 3 7 2 4 7

7. TYPE OF APPLICANT: (enter appropriate letter in box) [I]

A. State; B. County; C. Municipal; D. Township; E. Interstate; F. Intermunicipal; G. Special District; H. Independent School Dist; I. State Controlled Institution of Higher Learning; J. Private University; K. Indian Tribe; L. Individual; M. Profit Organization; N. Other (Specify): _____

8. TYPE OF APPLICATION: [X] New [ ] Continuation [ ] Revision

If Revision, enter appropriate letter(s) in box(es): [ ] [ ]

A. Increase Award B. Decrease Award C. Increase Duration D. Decrease Duration Other (Specify): _____

9. NAME OF FEDERAL AGENCY: **CALFED Bay Delta Program**

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER: [ ][ ] • [ ][ ][ ]

CALFED: Bay Delta Ecosystems Restoration Projects

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:

Genetic Identification of Watershed-Dependent Species of Special Concern in the Central Valley

12. AREAS AFFECTED BY PROJECT (cities, counties, states, etc.): **Entire CALFED region**

13. PROPOSED PROJECT: Start Date	Ending Date	14. CONGRESSIONAL DISTRICTS OF: a. Applicant	b. Project
1-Jan-01	31-Dec-03	12th	Various

15. ESTIMATED FUNDING:	
a. Federal	$345,312.00
b. Applicant	.00
c. State	.00
d. Local	.00
e. Other	.00
f. Program Income	.00
g. TOTAL	$345,312.00

16. IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES. THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON: DATE: _____

b. [X] PROGRAM IS NOT COVERED BY E.O. 12372

[ ] OR PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. IS THE APPLICANT DELINQUENT ON ANY FEDERAL DEBT?

[ ] Yes If "Yes" attach an explanation. No [X]

18. TO THE BEST OF MY KNOWLEDGE AND BELIEF, ALL DATA IN THIS APPLICATION/PREAPPLICATION ARE TRUE AND CORRECT. THE DOCUMENT HAS BEEN DULY AUTHORIZED BY THE GOVERNING BODY OF THE APPLICANT AND THE APPLICANT WILL COMPLY WITH THE ATTACHED ASSURANCES IF THE ASSISTANCE IS AWARDED

a. Typed Name of Authorized Representative	b. Title	c. Telephone number
Bruce Macher	Associate Dean, ORSP	(415) 338-7091

d. Signature of Authorized Representative: Bruce A. Macher

e. Date Signed: 5/12/00

Previous Editions Not Usable

Standard Form 424 (REV 4-88)

Prescribed by OMB Circular A-102

Authorized for Local Reproduction

OMB Approval No. 0348-0043

BUDGET INFORMATION – Non-Construction Programs

SECTION A – BUDGET SUMMARY

Grant Program Function or Activity (a)	Catalog of Federal Domestic Assistance Number (b)	Estimated Unobligated Funds		New or Revised Budget		
		Federal (c)	Non-Federal (d)	Federal (e)	Non-Federal (f)	Total (g)
1. SFSU		$851,669				$851,669
2						
3						
4						
5. TOTALS		$851,669		$0	$0	$851,669

SECTION B – BUDGET CATEGORIES

6. Object Class Categories	GRANT PROGRAM, FUNCTION OR ACTIVITY				Total
	(1)	(2)	(3)	(4)	(5)
a. Personnel	$33,500	$35,175	36934		$105,609
b. Fringe	$12,730	$13,367	$14,035		$40,132
c. Travel					$0
d. Equipment					$0
e. Supplies					$0
f. Contractual	$157,793	$163,374	$111,283		$432,450
g. Construction					$0
h. Other	$65,000	$25,000	$25,000		$115,000
i. Total Direct Charges (sum of 6a-6h)	$269,023	$236,916	$187,252	0	$693,191
j. Indirect Charges	$76,289	$43,445	$38,744		$158,478
k. TOTALS (sum of 6i and 6j)	$345,312	$280,361	$225,996		$851,669
7. Program Income					

Standard Form 424A (4-88)

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BUDGET INFORMATION – Non-Construction Programs

SECTION C – NON-FEDERAL RESOURCES

(a) Grant Program	(b) Applicant	(c) State	(d) Other Sources	(e) TOTALS
8.				
9.				
10.				
11.				
12. TOTALS (sum of lines 8 and 11)				

SECTION D – FORECASTED CASH NEEDS

	Total for 1st Year	1st Quarter	2nd Quarter	3rd Quarter	4th Quarter
13. Federal	$345,312	$86,328	$86,328	$86,328	$86,328
14. Non Federal					
15. TOTAL (sum of lines 13 and 14)	$345,312				

SECTION E – BUDGET ESTIMATES OF FEDERAL FUNDS NEEDED FOR BALANCE OF THE PROJECT

(a) Grant Program	FUTURE FUNDING PERIODS (Years) (b) First	(c) Second	(d) Third	(e) Fourth
16. SFSU	$345,312	$280,361	$225,996	
17.				
18.				
19.				
20. TOTALS (sum of lines 16 - 19)	$345,312	$280,361	$225,996	

SECTION F - OTHER BUDGET INFORMATION
(Attach additional Sheets if Necessary)

21. Direct Charges:	$693,191	22. Indirect Charges:	158,478

23. Remarks See attached detail budget.

CERTIFICATIONS REGARDING LOBBYING; DEBARMENT, SUSPENSION AND OTHER RESPONSIBILITY MATTERS; AND DRUG-FREE WORKPLACE REQUIREMENTS

Applicants should refer to the regulations cited below to determine the certification to which they are required to attest. Applicants should also review the instructions for certification included in the regulations before completing this form. Signature of this form provides for compliance with certification requirements under 34 CFR Part 82, "New Restrictions on Lobbying," and 34 CFR Part 85, "Government-wide Debarment and Suspension (Nonprocurement) and Government-wide Requirements for Drug-Free Workplace (Grants)." The certifications shall be treated as a material representation of fact upon which reliance will be placed when the Department of Education determines to award the covered transaction, grant, or cooperative agreement.

1. LOBBYING

As required by Section 1352, Title 31 of the U.S. Code, and implemented at 34 CFR Part 82, for persons entering into a grant or cooperative agreement over $100,000, as defined at 34 CFR Part 82, Sections 82.105 and 82.110, the applicant certifies that:

(a) No Federal appropriated funds have been paid or will be paid, by or on behalf of the undersigned, to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with the making of any Federal grant, the entering into of any cooperative agreement, and the extension, continuation, renewal, amendment, or modification of any Federal grant or cooperative agreement;

(b) If any funds other than Federal appropriated funds have been paid or will be paid to any person for influencing or attempting to influence an officer or employee of any agency, a Member of Congress, an officer or employee of Congress, or an employee of a Member of Congress in connection with this Federal grant or cooperative agreement, the undersigned shall complete and submit Standard Form - LLL, "Disclosure Form to Report Lobbying," in accordance with its instructions;

(c) The undersigned shall require that the language of this certification be included in the award documents for all subawards at all tiers (including subgrants, contracts under grants and cooperative agreements, and subcontracts) and that all subrecipients shall certify and disclose accordingly.

2. DEBARMENT, SUSPENSION, AND OTHER RESPONSIBILITY MATTERS

As required by Executive Order 12549, Debarment and Suspension, and implemented at 34 CFR Part 85, for prospective participants in primary covered transactions, as defined at 34 CFR Part 85, Sections 85.105 and 85.110--

A. The applicant certifies that it and its principals:

(a) Are not presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from covered transactions by any Federal department or agency;

(b) Have not within a three-year period preceding this application been convicted of or had a civil judgement rendered against them for commission of fraud or a criminal offense in connection with obtaining, attempting to obtain, or performing a public (Federal, State, or local) transaction or contract under a public transaction; violation of Federal or State antitrust statutes or commission of embezzlement, theft, forgery, bribery, falsification or destruction of records, making false statements, or receiving stolen property;

(c) Are not presently indicted for or otherwise criminally or civilly charged by a governmental entity (Federal, State, or local) with commission of any of the offenses enumerated in paragraph (2)(b) of this certification; and

(d) Have not within a three-year period preceding this application had one or more public transaction (Federal, State, or local) terminated for cause or default; and

B. Where the applicant is unable to certify to any of the statements in this certification, he or she shall attach an explanation to this application.

3. DRUG-FREE WORKPLACE (GRANTEES OTHER THAN INDIVIDUALS)

As required by the Drug-Free Workplace Act of 1988, and implemented at 34 CFR Part 85, Subpart F, for grantees, as defined at 34 CFR Part 85, Sections 85.605 and 85.610 -

A. The applicant certifies that it will or will continue to provide a drug-free workplace by:

(a) Publishing a statement notifying employees that the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance is prohibited in the grantee's workplace and specifying the actions that will be taken against employees for violation of such prohibition;

(b) Establishing an on-going drug-free awareness program to inform employees about:

(1) The dangers of drug abuse in the workplace;

(2) The grantee's policy of maintaining a drug-free workplace;

(3) Any available drug counseling, rehabilitation, and employee assistance programs; and

(4) The penalties that may be imposed upon employees for drug abuse violations occurring in the workplace;

(c) Making it a requirement that each employee to be engaged in the performance of the grant be given a copy of the statement required by paragraph (a);

(d) Notifying the employee in the statement required by paragraph (a) that, as a condition of employment under the grant, the employee will:

(1) Abide by the terms of the statement; and

(2) Notify the employer in writing of his or her conviction for a violation of a criminal drug statute occurring in the workplace no later than five calendar days after such conviction;

(e) Notifying the agency, in writing, within 10 calendar days after receiving notice under subparagraph (d)(2) from an employee or otherwise receiving actual notice of such conviction. Employers of convicted employees must provide notice, including position title, to: Director, Grants Policy and Oversight Staff, U.S. Department of Education, 400 Maryland Avenue, S.W. (Room 3652, GSA Regional Office Building No. 3), Washington, DC 20202-4248. Notice shall include the identification number(s) of each affected grant;

(f) Taking one of the following actions, within 30 calendar days of receiving notice under subparagraph (d)(2), with respect to any employee who is so convicted:

(1) Taking appropriate personnel action against such an employee, up to and including termination, consistent with the requirements of the Rehabilitation Act of 1973, as amended; or

(2) Requiring such employee to participate satisfactorily in a drug abuse assistance or rehabilitation program approved for such purposes by a Federal, State, or local health, law enforcement, or other appropriate agency;

(g) Making a good faith effort to continue to maintain a drug-free workplace through implementation of paragraphs (a), (b), (c), (d), (e), and (f).

B. The grantee may insert in the space provided below the site(s) for the performance of work done in connection with the specific grant:

Place of Performance (Street address. city, county, state, zip code)

SFSU

1600 Holloway Ave.

San Francisco, CA 94132

Check [ ] if there are workplaces on file that are not identified here.

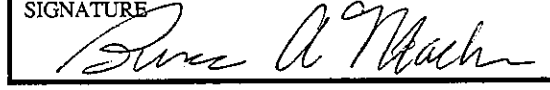
DRUG-FREE WORKPLACE
(GRANTEES WHO ARE INDIVIDUALS)

As required by the Drug-Free Workplace Act of 1988, and implemented at 34 CFR Part 85, Subpart F, for grantees, as defined at 34 CFR Part 85, Sections 85.605 and 85.610-

A. As a condition of the grant, I certify that I will not engage in the unlawful manufacture, distribution, dispensing, possession, or use of a controlled substance in conducting any activity with the grant; and

B. If convicted of a criminal drug offense resulting from a violation occurring during the conduct of any grant activity, I will report the conviction, in writing, within 10 calendar days of the conviction, to: Director, Grants Policy and Oversight Staff, Department of Education, 400 Maryland Avenue, S.W. (Room 3652, GSA Regional Office Building No. 3), Washington, DC 20202-4248. Notice shall include the identification number(s) of each affected grant.

As the duly authorized representative of the applicant, I hereby certify that the applicant will comply with the above certifications.

NAME OF APPLICANT	PR/AWARD NUMBER AND / OR PROJECT NAME
San Francisco State University	CALFED
PRINTED NAME AND TITLE OF AUTHORIZED REPRESENTATIVE Bruce Macher, Associate Dean, Research	
SIGNATURE 	DATE 5/12/00

Certification Regarding Debarment, Suspension, Ineligibility and Voluntary Exclusion -- Lower Tier Covered Transactions

This certification is required by the Department of Education regulations implementing Executive Order 12549, Debarment and Suspension, 34 CFR Part 85, for all lower tier transactions meeting the threshold and tier requirements stated at Section 85.110.

Instructions for Certification

1. By signing and submitting this proposal, the prospective lower tier participant is providing the certification set out below.

2. The certification in this clause is a material representation of fact upon which reliance was placed when this transaction was entered into. If it is later determined that the prospective lower tier participant knowingly rendered an erroneous certification, in addition to other remedies available to the Federal Government, the department or agency with which this transaction originated may pursue available remedies, including suspension and/or debarment.

3. The prospective lower tier participant shall provide immediate written notice to the person to which this proposal is submitted if at any time the prospective lower tier participant learns that its certification was erroneous when submitted or has become erroneous by reason of changed circumstances.

4. The terms "covered transaction," "debarred," "suspended," "ineligible," "lower tier covered transaction," "participant," " person," "primary covered transaction," " principal," "proposal," and "voluntarily excluded," as used in this clause, have the meanings set out in the Definitions and Coverage sections of rules implementing Executive Order 12549. You may contact the person to which this proposal is submitted for assistance in obtaining a copy of those regulations.

5. The prospective lower tier participant agrees by submitting this proposal that, should the proposed covered transaction be entered into, it shall not knowingly enter into any lower tier covered transaction with a person who is debarred, suspended, declared ineligible, or voluntarily excluded from participation in this covered transaction, unless authorized by the department or agency with which this transaction originated.

6. The prospective lower tier participant further agrees by submitting this proposal that it will include the clause titled □Certification Regarding Debarment, Suspension, Ineligibility, and Voluntary Exclusion-Lower Tier Covered Transactions,□□without modification, in all lower tier covered transactions and in all solicitations for lower tier covered transactions.

7. A participant in a covered transaction may rely upon a certification of a prospective participant in a lower tier covered transaction that it is not debarred, suspended, ineligible, or voluntarily excluded from the covered transaction, unless it knows that the certification is erroneous. A participant may decide the method and frequency by which it determines the eligibility of its principals. Each participant may but is not required to, check the Nonprocurement List.

8. Nothing contained in the foregoing shall be construed to require establishment of a system of records in order to render in good faith the certification required by this clause. The knowledge and information of a participant is not required to exceed that which is normally possessed by a prudent person in the ordinary course of business dealings.

9. Except for transactions authorized under paragraph 5 of these instructions, if a participant in a covered transaction knowingly enters into a lower tier covered transaction with a person who is suspended, debarred, ineligible, or voluntarily excluded from participation in this transaction, in addition to other remedies available to the Federal Government, the department or agency with which this transaction originated may pursue available remedies, including suspension and/or debarment.

Certification

(1) The prospective lower tier participant certifies, by submission of this proposal, that neither it nor its principals are presently debarred, suspended, proposed for debarment, declared ineligible, or voluntarily excluded from participation in this transaction by any Federal department or agency.

(2) Where the prospective lower tier participant is unable to certify to any of the statements in this certification, such prospective participant shall attach an explanation to this proposal.

NAME OF APPLICANT	PR/AWARD NUMBER AND/OR PROJECT NAME
San Francisco State University	CALFED
PRINTED NAME AND TITLE OF AUTHORIZED REPRESENTATIVE	
Bruce Macher, Associate Dean, Research	
SIGNATURE	DATE
Bruce A. Macher	5/12/00

ED 80-0014, 9/90 (Replaces GCS-009 (REV.12/88), which is obsolete)

OMB Approval No. 0348-0040

ASSURANCES - NON-CONSTRUCTION PROGRAMS

PLEASE DO NOT RETURN YOUR COMPLETED FORM TO THE OFFICE OF MANAGEMENT AND BUDGET. SEND IT TO THE ADDRESS PROVIDED BY THE SPONSORING AGENCY.

Note: Certain of these assurances may not be applicable to your project or program. If you have questions, please contact the awarding agency. Further, certain Federal awarding agencies may require applicants to certify to additional assurances. If such is the case, you will be notified.

As the duly authorized representative of the applicant I certify that the applicant:

1. Has the legal authority to apply for Federal assistance, and the institutional, managerial and financial capability (including funds sufficient to pay the non-Federal share of project cost) to ensure proper planning, management, and completion of the project described in this application.

2. Will give the awarding agency, the Comptroller General of the United States, and if appropriate, the State, through any authorized representative, access to and the right to examine all records, books, papers, or documents related to the award; and will establish a proper accounting system in accordance with generally accepted accounting standards or agency directives.

3. Will establish safeguards to prohibit employees from using their positions for a purpose that constitutes or presents the appearance of personal or organizational conflict of interest, or personal gain.

4. Will initiate and complete the work within the applicable time frame after receipt of approval of the awarding agency.

5. Will comply with the Intergovernmental Personnel Act of 1970 (42 U.S.C. 4728-4763) relating to prescribed standards for merit systems for programs funded under one of the 19 statutes or regulations specified in Appendix A of OPM's Standards for a Merit System of Personnel Administration (5 C.F.R. 900, Subpart F).

6. Will comply with all Federal statutes relating to nondiscrimination. These include but are not limited to: (a) Title VI of the Civil Rights Act of 1964 (P.L. 88-352) which prohibits discrimination on the basis of race, color or national origin; (b) Title IX of the Education Amendments of 1972, as amended (20 U.S.C. 1681-1683, and 1685-1686), which prohibits discrimination on the basis of sex; (c) Section 504 of the Rehabilitation Act of 1973, as amended (29 U.S.C. 794), which prohibits discrimination on the basis of handicaps; (d) the Age Discrimination Act of 1975, as amended (42 U.S.C. 6101-6107), which prohibits discrimination on the basis of age; (e) the Drug Abuse Office and Treatment Act of 1972 (P.L. 92-255), as amended, relating to nondiscrimination on the basis of drug abuse; (f) the Comprehensive Alcohol Abuse and Alcoholism Prevention, Treatment and Rehabilitation Act of 1970 (P.L. 91-616), as amended, relating to nondiscrimination on the basis of alcohol abuse or alcoholism; (g) 523 and 527 of the Public Health Service Act of 1912 (42.U.S.C. 290 dd-3 and 290 ee 3), as amended, relating to confidentiality of alcohol and drug abuse patient records; (h) Title VIII of the Civil Rights Act of 1968 (42 U.S.C. 3601 et seq.), as amended, relating to nondiscrimination in the sale, rental or financing of housing; (i) any other nondiscrimination provisions in the specific statute(s) under which application for Federal assistance is being made; and (j) the requirements of any other nondiscrimination statute(s) which may apply to the application.

7. Will comply, or has already complied, with the requirements of Titles II and III of the uniform Relocation Assistance and Real Property Acquisition Policies Act of 1970 (P.L. 91-646) which provide for fair and equitable treatment of persons displaced or whose property is acquired as a result of Federal or federally assisted programs. These requirements apply to all interests in real property acquired for project purposes regardless of Federal participation in purchases.

8. Will comply, as applicable, with the provisions of the Hatch Act (5 U.S.C. 1501-1508 and 7324-7328) which limit the political activities of employees whose principal employment activities are funded in whole or in part with Federal funds.

9. Will comply, as applicable, with the provisions of the Davis-Bacon Act (40 U.S.C. 276a to 276a-7), the Copeland Act (40 U.S.C. 276c and 18 U.S.C. 874) and the Contract Work Hours and Safety Standards Act (40 U.S.C. 327-333), regarding labor standards for federally assisted construction subagreements.

10. Will comply, if applicable, with flood insurance purchase requirements of Section 102(a) of the Flood Disaster Protection Act of 1973 (P.L. 93-234) which requires recipients in a special flood hazard area to participate in the program and to purchase flood insurance if the total cost of insurable construction and acquisition is $10,000 or more.

11. Will comply with environmental standards which may be prescribed pursuant to the following: (a) institution of environmental quality control measures under the National Environmental Policy Act of 1969 (P.L. 91-190) and Executive Order (EO) 11514; (b) notification of violating facilities pursuant to EO 11738; (c) protection of wetlands pursuant to EO 11990; (d) evaluation of flood hazards in floodplains in accordance with EO 11988; (e) assurance of project consistency with the approved State management program developed under the Coastal Zone Management Act of 1972 (16 U.S.C. 1451 et seq.); (f) conformity of Federal actions to State (Clear Air) Implementation Plans under Section 176(c) of the Clear Air Act of 1955, as amended (42 U.S.C. 7401 et seq.); (g) protection of underground sources of drinking water under the Safe Drinking Water Act of 1974, as amended, (P.L. 93-523); and (h) protection of endangered species under the Endangered Species Act of 1973, as amended, (P.L. 93-205).

12 Will comply with the Wild and Scenic Rivers Act of 1968 (16 U.S.C. 1721 et seq.) related to protecting components or potential components of the national wild and scenic rivers system.

13. Will assist the awarding agency in assuring compliance with Section 106 of the National Historic Preservation Act of 1966, as amended (16 U.S.C. 470), EO 11593 (identification and protection of historic properties), and the Archaeological and Historic Preservation Act of 1974 (16 U.S.C. 469 a-1 et seq.).

14. Will comply with P.L. 93-348 regarding the protection of human subjects involved in research, development, and related activities supported by this award of assistance.

15. Will comply with the Laboratory Animal Welfare Act of 1966 (P.L. 89-544, as amended, 7 U.S.C. 2131 et seq.) pertaining to the care, handling, and treatment of warm blooded animals held for research, teaching, or other activities supported by this award of assistance.

16. Will comply with the Lead-Based Paint Poisoning Prevention Act (42 U.S.C. 4801 et seq.) which prohibits the use of lead- based paint in construction or rehabilitation of residence structures.

17. Will cause to be performed the required financial and compliance audits in accordance with the Single Audit Act Amendments of 1996 and OMB Circular No. A-133, Audits of States, Local Governments, and Non-Profit Organizations.

18. Will comply with all applicable requirements of all other Federal laws, executive orders, regulations and policies governing this program.

SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL	TITLE
Bruce Macher, Associate Dean, Research	CALFED
APPLICANT ORGANIZATION	DATE SUBMITTED
San Francisco State University	5/12/00

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